THE FEASIBILITY OF USING THE MIE THEORY FOR THE SCATTERING OF LIGHT FROM
SUSPENSIONS OF SPHERICAL BACTERIA

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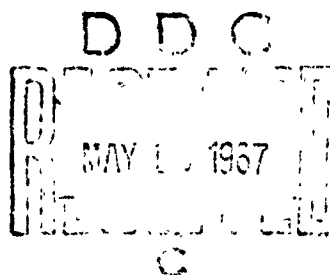# THE FEASIBILITY OF USING THE MIE THEORY FOR THE SCATTERING OF LIGHT FROM SUSPENSIONS OF SPHERICAL BACTERIA

Recently in the literature, attempts have been made to examine the results of the photometric study of bacterial suspensions from the positions of the physical theory of scattering. Thus, Lewis and Lothian [1], by using the approximate equation of Hulst, determined the refractive index of spores; Beigter and Gunter [2], while studying the growth of synchronous cultures of staphylococcus, used the theory of scattering in the approximation of Debye; Koga and Fujita [3] used the theory of Rayleigh--Gans for determining the average dimension of bacteria and their refractive index. However, an analysis of the last magnitudes together with certain data by Koch [4], also obtained by using the Rayleigh-Gans theory, show that they differ substantially from the real values which are characteristic for these magnitudes. As an example, according to the data by Koch, for the refractive index of bacteria the Rayleigh-Gans theory gives a value of 1.6, which is known to be an overstated value, since the refractive index for dry protein does not exceed values of 1.52--1.53. Besides this, as was demonstrated by direct measurements by the method of immersion refractometry [5], the refractive index of bacteria is found in the limts of 1.370--1.400. Similar disparities also take place in the work by Kaga and

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Fujita. This makes it possible to suggest that the Rayleigh--Gans Theory does not always satisfactorily describe the light scattering of bacterial suspensions.

As is known, the Rayleigh--Gans theory is correct only for a small interval of partical dimensions, which depends on their refractive index. The Mie theory[6] is a more complete and strict theory, describing the scattering of light of spherical particles of any diameter and refractive index. It was verified by numerous investigators [7] on suspensions of various latexes and solutions of colloidal particles. It was shown to be completely accurate relative to these systems.

However, suspensions of bacteria differ from such systems by a number of peculiarities. The most important of these is the fact that each particle of the dispersed phase (cell) of these coarsely dispersed suspensions represents a colloidal microsystem made up of organic substances in a saline solution, which based on refractive index is close to the external dispersion medium. The cells are delimited from the latter anatomically by a cell wall of specific thickness with a higher refractive index than the protoplasm of the cell and the medium surrounding it.

The stated peculiarities make the feasibility of using the Mie theory for the light scattering of bacterial suspensions vague. Together with this, the solving of the problem concerning the scattering of visible light by these systems has a great theoretical and practical importance in microbiology. Thanks to simplicity and convenience, the photometric method has found extensive application in microbiology, but the current equations, describing the light scattering of suspensions of bacteria [8], bear a purely empirical nature and are not always correct and far from completely

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reflect the substance of the processes which take place during this phenomenon.

In connection with this we have undertaken the present work for the purpose of resolving the problem concerning the feasibility of applying the Mie theory to the light scattering of bacterial suspensions. In this we have used both our own experimental material as well as data from the literature.

As is known, suspensions of bacteria are systems which hardly absorb light in the visible range of the spectrum. As a result of this, the attenuation factor τ in the law of Lambert-Beer $I = I_0 \cdot e^{-\tau x}$ (where I_0 -- is the intensity of incident light, I -- the intensity of transmitted light, x -- the path length of a ray in a scattering system) is completely determined by the scattering and equals the number of particles in a unit of volume N , multiplied by the amount of energy R , scattered by each particle:

$$\tau = NR .$$ (1)

For R , which is also called the cross section of scattering, the Mie theory gives the expression:

$$R = K_s \pi r^2 ,$$ (2)

where r -- the radius of a particle, K_s -- the dimensionless coefficient of scattering, which is determined in the following manner:

$$K_s = \frac{2}{a^2} \sum_1^\infty \frac{|a_\nu|^2 + |b_\nu|^2}{2\nu + 1} = \frac{1}{a^2} \int_0^\pi (i_1 + i_2) \sin \gamma d\gamma,$$ (3)

here $a = \frac{2\pi r}{\lambda_M}$, where λ_M -- the wave length of light in a medium with a refractive index n_0 , a_ν and b_ν -- the ν components of the electric and magnetic fields, i_1 and i_2 -- the intensities of the light components, polarized in mutually perpendicular directions.

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The scattering coefficient is expressed through the Bessel functions and Legendre's associated polynomials, and therefore the obtaining of numerical data from the equation (3) is very difficult. However, at the present time there are several tables which have been compiled for the various dimensions of particles and refractive indices [9].

From equations (1) and (2) it follows that

$$\tau = K_s \pi r^2 N.$$
(4)

If now advantage is taken of the weight concentration of the particles

$$C = \frac{4}{3} \pi r^3 \delta N,$$

where δ -- is the density of the particles, it is possible from here to arrive at the expression

$$\frac{K_s}{d} = \frac{D}{C} \cdot \frac{2.3 \cdot \delta \cdot \lambda_m}{3 \pi r},$$
(5)

where D -- is the optical density, equal to $\frac{\tau x}{2.3}$. Here the concentrations C should be sufficiently small in order to omit secondary scattering.

We will use the equation (4) when analyzing data from the literature; for the treatment of experimental results the expression (5) is more suitable.

Materials and Methods

In the experimental work we used native suspensions of three strains of spherical bacteria Staphylococcus aureus (1; 209; 219).
The dimension of the cells was determined in a phase contrast microscope by the method of scale microphotography. The refractive index of these bacteria, determined by the method of immersion refractometry [5], was equal or very close to 1.400. This satisfies the values $m = \frac{n}{n_0}$, where

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n -- is the refractive index of the cells, which are equal or differ little from 1.05, as a result of which, during the calculations, we used the tables of Heller, Pangonis and Jacobson [10], compiled for m =1.05.

The experiments consisted of a comparison of the dimensions of the bacteria, determined under a microscope and calculated from tests on the measurement of the optical density of suspensions of these bacteria according to the equation (5). In this equation all the values to the right are determined experimentally. The values $\frac{K_s}{a}$ found in this manner are equal to the theoretical [ones] from the table, made up according to the equation (3). Then, based on the value $\frac{K_s}{a}$ the dimensions of the cells are calculated.

The measurements were made on suspensions prepared from 24-hour cultures of bacteria grown on solid nutrient medium. For determining the concentration, a suspension of the bacterial mass from the nutrient medium was added to a known amount of physiological solution. Following a thorough mixing the optical density of the suspensions was measured. The determinations were on a SF-4 spectrophotometer and a FEK-54 photocolorimeter at various wave lengths within the limits of the visible spectrum. In the case of measurements on the SF-4, a special diaphragm was used in order to reduce to a minimum the amount of scattered light reaching the photoelement. The size of the diaphragm aperture was selected in such a manner that a further lessening of it hardly affected the results of the measurements. In both cases (on the SF-4 and the FEKN-54) similar results were obtained, however, on the photocolorimeter they were less accurate because of fully understandable reasons (the use of light filters in place of a

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monochromator, small distance between the scattering system and the photoelement). The results of the measurements on the SF-4 are presented in figure 1.

When calculating the dimensions for these bacteria based on this data along with the use of the Mie equation, analogous results were obtained for all the bacteria used. The greatest conformity of calculated and measured values was observed for bacteria of the strain St. aureus-1. These data are presented in table 1. For the remaining bacteria the deviations of the calculated values from the measured values was somewhat greater. This is explained by the fact that for them the values m differed somewhat from the tabular value 1.05.

Discussion of Results

As is seen from table 1, the conformity of the dimensions, determined under the microscope and calculated according to the Mie equation, are completely satisfactory for all the wave lengths of the visible spectrum which were used. The average relative error for the entire spectrum is found within the limits of 3%. This fact may serve as proof that the scattering of light by spherical bacteria obeys the equations of the Mie theory. In view of the above stated peculiarities of such systems, it is evidently possible that for bacterial cells the relationship of additivity of refractive indices of a suspension is easily satisfied:

$$n_1 V_1 = n_2 V_2 + n_3 V_3 , \qquad (6)$$

where n_1 , n_2 , n_3 -- are the refractive indices of the system (cell), dry substance and the dispersion medium correspondingly; V_1 , V_2 , V_3

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are their volumes. As a result of this the cell may be viewed as a homo-
geneous particle with a refractive index, obtained according to the
equation (6) from the indices of refraction of the intracellular saline
solution and the dry substances.

The feasibility of using the Mie theory for the light scattering of
suspensions of spherical bacteria may be illustrated also by the data from
the literature.

Thus, many authors [8; 11; 12] who have studied the light scattering
of spherical bacteria have shown that the optical density is directly
proportional to the concentration of cells and the cellular surface S :

$$D = Z \cdot N \cdot S, \qquad (7)$$

where Z -- is the constant multiple.

In one of the works [8] it was shown that for a great number of strains
this coefficient of proportionality equals 0.016. For this the dimensions
of the cells of the strains which were used are presented in table 2. The
refractive index of these bacteria is close to 1.400.

By using the Mie theory, it is easy to arrive at the formula(7).
Actually, the conditions under which it was obtained were as follows: the
diameter of the particles was from 0.82 to 1.12 microns; the wave length
equaled 0.540 microns and the length ofthe optical path comprised 0.5 cm.
These data correspond to values of a from 6.4 to 8.9. From the tables
for the Mie equation we find that the scattering coefficients which
correspond to them are equal to 0.198 and 0.393, while the average value
$\overline{K}_s = 0.296.$ A comparison of the equations (4), obtained from the Mie
theory, and (7), found experimentally, produces: $Z = \dfrac{0.296 \cdot 05}{4 \cdot 2.3}$, from
where $Z = 0.016$, that is, a complete conformity is observed between
the theory and experimental data.

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Along with this, this calculation shows that the empirical formula (7) is correct only under strictly specific conditions, but even in this case it is inaccurate and average, while at the same time the Mie theory makes it possible to accurately calculate the scattering coefficient for each species of bacteria in each concrete situation. This, in its turn, makes it possible to accurately determine such parameters as the concentration of cells in a suspension, the dimensions of bacteria and their refractive index.

As regards empirical investigations of the light scattering of bacterial suspensions and functions of the type (7), still one more circumstance must be noted. In explaining a similar nature of dependencies, several authors [8; 11] note that it is the result of the fact that the scattering of light insuch systems is conditioned mainly by its reflection from the cellular surface, as a result of which there is a proportionality of the optical density to this parameter. As can be seen from the calculation presented above, this proportionality of the cellular surface emerges as a result of the fact that the turbidity is directly proportional to the cross section of scattering R, which in its turn is connected linerly with the geometric cross section of the particle. But this latter, based on a known theorem, [13] is equal to one fourth of the surface of the particle.

In conclusion we will dwell on the reason for those results which were obtained in the above mentioned works by Koga and Fujita [3] and Koch [4], who used the Rayleigh--Gans theory of scattering.

As is known, the Rayleigh--Gans theory for spherical particles gives the expression for the scattering coefficient in the form:

$$K_s = \frac{1}{2} \rho^2 ,$$

(8)

where $\rho = 2a(m-1),$

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The application of this theory has fully specific criteria $\underline{!}$

a) the phase shift of the ray during its passage through the particle should be small, that is $\rho \leqslant 1$,

b) for small $\alpha's$, this means that $m \to 1$,

c) the value of the scattering coefficient itself is small: $K_s \ll 1$.

For a comparison, figure 2 presents the scattering coefficient curves, calculated by us according to the Rayleigh-Gans equation (8), based on the Hulst equation, and taken from the tables according to the Mie equation for $m = 1.05$ ($n = 1.400$, $n_0 = 1.333$). As can be seen from the drawing, the Rayleigh-Gans theory is applicable on a sector up to $\alpha \leqslant 8$. In a cited work [3], for a yeast cell the authors have: $2r = 5.6\mu$, $\lambda = 1\mu$, $m = 1.05$. It can be easily seen that these facts correspond to $\alpha = 23.5$ and $\rho = 2.35$, that is, the criterion for the applicability of the Rayleigh-Gans theory is not fulfilled. Thus, as a result of this the scattering coefficient is not at all $\ll 1$, as required by the Rayleigh-Gans theory, but is equal to 2.79, along with 2.12 according to the Mie theory. If it concerns the approximations of the Mie theory, then in the stated case it naturally follows to utilize the approximations of Hulst [13]

$$ K_s = 2 - 4 \cdot \frac{\sin \rho}{\rho} + 4 \cdot \frac{(1 - \cos \rho)}{\rho^2}, $$

which gives the scattering coefficient equal to 2.05, and this is much closer to true. Thus, the application of the Rayleigh-Gans theory in the stated case was incorrect.

We will point out still one more criterion for the applicability of the Rayleigh-Gans theory which may be easily determined experimentally. For this there is no requirement for knowledge of the dimension of the

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scattering particles or of their refractive index. It can be seen from

figure 2 that with an increase of a the slope of the curve $K_s(a)$ changes

monotonically. Consequently the function $\frac{d \ln K_s}{d \ln a} = y$ may serve as a

single-valued index of the dimension of the particles. But the value

of this function is easily found based on the measurement of turbidity or

optical density with two arbitrary different wave lengths, since it can

be shown that

$$y = \frac{d \ln D}{d \ln \lambda} = \frac{\Delta \lg D}{\Delta \lg \lambda} = \frac{\lg D_{\lambda_2} - \lg D_{\lambda_1}}{\lg \lambda_1 - \lg \lambda_2} \quad .$$

These equations were used for the practical determination of the degree

of dispersion of various bacterial suspensions [14] and solutions of

latexes [15].

As it follows from the equation of Rayleigh-Gans, the value of y in

this approximation should satisfy the condition $y \geqslant 2$, while with $y = 4$ there

takes place a purely Rayleigh scattering, which holds true for particles

with a dimension much less than the wave length. Values of y less than

2 will indicate that the stated system is not subordinated to the Rayleigh-

Gans scattering. Namely such values of $y < 2$ are often observed for bacterial

suspensions, which is testified to by table 3 (partially taken from the

work [14]) from which it clearly follows that the theory of Rayleigh-Gans

is not applicable to many suspensions of bacteria, and in these cases it

is necessary to utilize the more general Mie theory. The applicability of

this theory to such systems has been demonstrated in the present work.

Conclusions

1. The distribution of the energy of light waves during the interaction

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with suspensions of spherical bacteria obeys the Mie theory of scattering.

2. In the visible range of the spectrum the Rayleigh-Gans theory cannot always be used for describing the scattering of light of bacterial suspensions.

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Table 1

Values of the dimensions of bacteria St. aureus-1, calculated according to the Mie equation for various wave lengths of the visible spectrum (the microscopically measured diameter of the cells equaled 0.82 mμ)

λ mμ	D	$\dfrac{K_s}{a}$	a	Diameter μ
450	1.29	0.0352	7.35	0.780
500	1.06	0.032	6.6	0.790
550	0.875	0.0291	6.1	0.801
600	0.726	0.0263	5.5	0.790
650	0.626	0.0217	5.2	0.807
700	0.542	0.0229	4.9	0.819

Average diameter   4.787:6= 0.798 μ

Table 2

Average diameters of cells of spherical bacteria (taken from /8/)

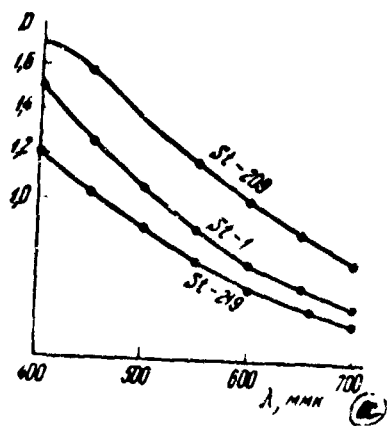| Name of strain		Average diameter, μ	Standard deviation	Average error
Staphyl aureus-1		0.82	0.092	0.005
"	" LA	0.83	0.091	0.005
"	" K-1	0.89	0.096	0.006
"	" Ya	0.97	0.092	0.005
"	" 75	1.04	0.0093	0.05
"	" 633	1.1	0.101	0.007
"	" S	1.12	0.098	0.006
"	" 4-1	1.16	0.093	0.005

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Figure 1. Dependency of the optical density of suspensions of various spherical bacteria on the wave length.
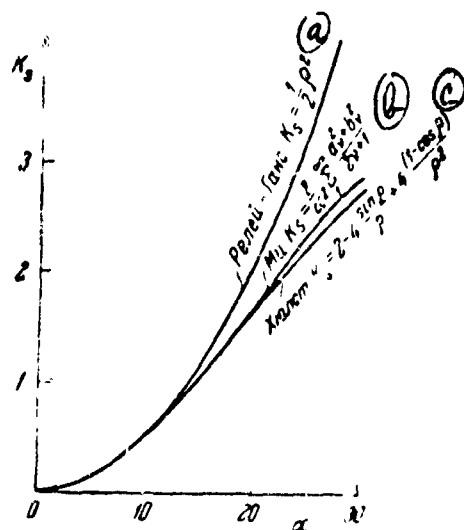
a -- $\lambda, m\mu$

Figure 2. Comparison of the Mie theory with the approximations of Rayleigh -Gans and Hulst.

a -- Rayleigh--Gans
b -- Mie
c -- Hulst

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Table 3

Values of the indices for the degree of dispersion y of suspensions

of various bacteria (partially from [14])

Microorganisms	Index of degree of dispersion	Microorganisms	Index of degree of dispersion
Pertussoid bacteria	2.11	E. coli	1.51
Staphylococcus aureus	2.02	Sarcina lutea	0.94
Staphylococcus albus	1.78	BCG	0.7
Typhous bacteria	1.58	Brucellosis bacteria	1.3
		Tularemia bacteria	2.0

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